Amendments to the Specification

Please amend the specification as shown:

Please delete paragraph [0016] and replace it with the following paragraph:

[0016] Yet another aspect of this invention is to provide a kit for administration of a botulinum toxin to a subject. The kit includes a device for delivering the botulinum toxin to the skin and a composition containing a carrier having a polymeric backbone with attached positively charged branching groups selected from $-(gly)_{n1}-(arg)_{n2}$ **(SEQ ID NO: 1)**, HIV-TAT and fragments thereof, and Antennapedia PTD, in which the subscript n1 is an integer of from 0 to about 20, and the subscript n2 is independently an odd integer of from about 5 to about 25.

Please amend paragraph [0027] as follows:

[0027] FIG. 8a represents the results of an experiment demonstrating efficiency of botulinum toxin therapeutically delivered across intact skin as a topical agent using a short peptidyl carrier for the treatment of axillary hyperhidrosis on human subjects. Graph depicts significant reduction in amount of sweat (mg per 5 minutes) measured gravimetrically 4 weeks after treatment with ~~Botox~~ BOTOX® plus a short peptidyl carrier or carrier alone. Results are 4 week values as ratio to baseline value for same group, with significance determined by Wilcoxon analysis with $P<0.05$. N=10 patients. FIG. 8b represents the results of an experiment demonstrating efficiency of botulinum toxin therapeutically delivered across intact skin as a topical agent using a short peptidyl carrier for the treatment of axillary hyperhidrosis on human subjects. Graph depicts significant reduction in amount of sweat (mg per 5 minutes) measured gravimetrically 4 weeks after treatment with ~~Botox~~ BOTOX® plus a short peptidyl carrier or carrier alone. Results are treatment values as ratio to control value for both timepoints, with significance determined by Wilcoxon analysis with $P<0.05$. N=10 patients.

Please amend paragraph [0028] as follows:

[0028] FIG. 9 shows photographs depicting Minor's starch/iodine test before and after treatment with "Essentia Botox lotion" topically for the treatment of axillary hyperhidrosis.

Starch/iodine test at Baseline vs. 2 week is shown where right axilla was treated with "Essentia Botox lotion" (a and c) and left axilla was applied with the control (b and d) for subject #12. These photographs illustrate typical benefits observed after treatment with carrier~~+ botox~~ BOTOX® in starch iodine. Although some crossover is observed on the control side (consistent with 25% reduction in gravimetric data), significant reductions are afforded with treatment (consistent with 65% reduction in gravimetric data on treated side).

Please delete paragraphs [0036] to [0037] and replace them with the following paragraphs:

[0036] In one embodiment, the positively charged backbone is a polypeptide having branching groups (also referred to as efficiency groups). As used herein, an efficiency group or branching group is any agent that has the effect of promoting the translocation of the positively charged backbone through a tissue or cell membrane. Non-limiting examples of branching or efficiency groups include $-(gly)_{n1}-(arg)_{n2}$ **(SEQ ID NO: 1)**, HIV-TAT or fragments thereof, or the protein transduction domain of Antennapedia, or a fragment thereof, in which the subscript n1 is an integer of from 0 to 20, more preferably 0 to 8, still more preferably 2 to 5, and the subscript n2 is independently an odd integer of from about 5 to about 25, more preferably about 7 to about 17, most preferably about 7 to about 13. Still further preferred are those embodiments in which the HIV-TAT fragment has the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$ **(SEQ ID NO: 2)**, $(gly)_p$-YGRKKRRQRRR-$(gly)_q$ **(SEQ ID NO: 3)** or $(gly)_p$-RKKRRQRRR-$(gly)_q$ **(SEQ ID NO: 4)** wherein the subscripts p and q are each independently an integer of from 0 to 20 and the fragment is attached to the backbone via either the C-terminus or the N-terminus of the fragment. Preferred HIV-TAT fragments are those in which the subscripts p and q are each independently integers of from 0 to 8, more preferably 2 to 5. In another preferred embodiment the positively charged side chain or branching group is the Antennapedia (Antp) protein transduction domain (PTD), or a fragment thereof that retains activity. Preferably the positively charged carrier includes side-chain positively charged branching groups in an amount of at least about 0.05%, as a percentage of the total carrier weight, preferably from about 0.05 to about 45 weight %, and most preferably from about 0.1 to about 30 weight %. For positively charged branching groups having the formula $-(gly)_{n1}-(arg)_{n2}$ **(SEQ ID NO: 1)**, the most preferred amount is from about 0.1 to about 25%.

[0037] In another embodiment, the backbone portion is a polylysine and positively charged branching groups are attached to the lysine sidechain amino groups. The polylysine may have a molecular weight of from about 10,000 to about 1,500,000, preferably from about 25,000 to about 1,200,000, and most preferably from about 100,000 to about 1,000,000. It can be any of the commercially available (Sigma Chemical Company, St. Louis, Mo., USA) polylysines such as, for example, polylysine having MW > 70,000, polylysine having MW of 70,000 to 150,000, polylysine having MW 150,000 to 300,000 and polylysine having MW > 300,000. The selection of an appropriate polylysine will depend on the remaining components of the composition and will be sufficient to provide an overall net positive charge to the composition and provide a length that is preferably from one to four times the combined length of the negatively charged components. Preferred positively charged branching groups or efficiency groups include, for example, -gly-gly-gly-arg-arg-arg-arg-arg-arg-arg (-Gly_3Arg_7) **(SEQ ID NO: 5)** or HIV-TAT. In another preferred embodiment the positively charged backbone is a long chain polyalkyleneimine such as a polyethyleneimine, for example, one having a molecular weight of about 1,000,000.

Please delete paragraphs [0039] to [0040] and replace them with the following paragraphs:

[0039] In one embodiment of the invention, only a positively charged carrier that has positively charged branching groups is necessary for transdermal delivery of the botulinum toxin. In certain embodiments, the positively charged carrier is a polypeptide (e.g., lysine, arginine, ornithine, homoarginine, and the like) having multiple positively charged side-chain groups, as described above. Preferably, the polypeptide has a molecular weight of at least about 10,000. In another embodiment, the positively charged carrier is a nonpeptidyl polymer such as a polyalkyleneimine having multiple positively charged side-chain groups having a molecular weight of at least about 100,000. Such polyalkyleneimines include polyethylene- and polypropyleneimines. In either instance, for use as the sole necessary agent for transdermal delivery the positively charged carrier molecule includes positively charged branching or efficiency groups, comprising -$(gly)_{n1}$-$(arg)_{n2}$ **(SEQ ID NO: 1)**, in which the subscript n1 is an integer of from 0 to 20 more preferably 0 to 8, still more preferably 2 to 5, and the subscript n2 is independently an odd integer of from about 5 to about 25, more preferably from about 7 to about

17, and most preferably from about 7 to about 13, HIV-TAT or fragments thereof, or Antennapedia PTD or a fragment thereof. Preferably the side-chain or branching groups have the general formula $-(gly)_{n1}-(arg)_{n2}$ **(SEQ ID NO: 1)** as described above. Other preferred embodiments are those in which the branching or efficiency groups are HIV-TAT fragments that have the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$ **(SEQ ID NO: 2)**, $(gly)_p$-YGRKKRRQRRR-$(gly)_q$ **(SEQ ID NO: 3)**, or $(gly)_p$-RKKRRQRRR-$(gly)_q$ **(SEQ ID NO: 4)**, wherein the subscripts p and q are each independently an integer of from 0 to 20 and the fragment is attached to the carrier molecule via either the C-terminus or the N-terminus of the fragment. The side branching groups can have either the D- or L-form (R or S configuration) at the center of attachment. Preferred HIV-TAT fragments are those in which the subscripts p and q are each independently integers of from 0 to 8, more preferably 2 to 5. Other preferred embodiments are those in which the branching groups are Antennapedia PTD groups or fragments thereof that retain the group's activity. These are known in the art, for instance, from Console et al., J. Biol. Chem. 278:35109 (2003). Preferably, the positively charged carrier includes side-chain positively charged branching groups in an amount of at least about 0.05%, as a percentage of the total carrier weight, preferably from about 0.05 to about 45 weight %, and most preferably from about 0.1 to about 30 weight %. For positively charged branching groups having the formula $-(gly)_{n1}-(arg)_{n2}$ **(SEQ ID NO: 1)**, the most preferred amount is from about 0.1 to about 25%.

[0040] In another embodiment, the carrier is a polylysine with positively charged branching groups attached to the lysine side-chain amino groups. The polylysine used in this particularly embodiment can be any of the commercially available (Sigma Chemical Company, St. Louis, Mo., USA, e.g.) polylysines such as, for example, polylysine having MW > 70,000, polylysine having MW of 70,000 to 150,000, polylysine having MW 150,000 to 300,000 and polylysine having MW > 300,000. However, preferably the polylysine has MW of at least about 10,000. Preferred positively charged branching groups or efficiency groups include, for example, -gly-gly-gly-arg-arg-arg-arg-arg-arg-arg ($-Gly_3Arg_7$) **(SEQ ID NO: 5)**, HIV-TAT or fragments of it, and Antennapedia PTD or fragments thereof.

Please amend paragraph [0047] as follows:

[0048] In particularly preferred embodiments, the compositions include gelling agents and/or viscosity-modifying agents. These agents are generally added to increase the viscosity of the

composition, so as to make the application of the composition easier and more accurate. Additionally, these agents help to prevent the aqueous botulinum toxin/carrier solution from drying out, which tends to cause a decrease in the activity of the botulinum toxin. Particularly preferred agents are those that are uncharged and do not interfere with the botulinum toxin activity or the efficiency of the toxin-carrier complexes in crossing skin. The gelling agents may be certain cellulose-based gelling agents, such as hydroxypropylcellulose (HPC) for example. In some embodiments, the botulinum toxin/carrier complex is formulated in a composition having 2-4% HPC. Alternatively, the viscosity of a solution containing a botulinum toxin/carrier complex may be altered by adding polyethylene glycol (PEG). In other embodiments, the botulinum toxin/carrier solution is combined with pre-mixed viscous agents, such as CETAPHIL~~Cetaphil~~® moisturizer.

Please amend paragraph [0049] as follows:

[0049] By way of example, if volume constraints require reconstituting 100 U of botulinum toxin in 0.5 ml of solution, rather than 2.5 ml, one typically observes that the botulinum toxin will exhibit undesirable aggregation, and thus lowered activity. However, by adding 1% EtOH as a dispersing agent, fully activity is maintained even after 24 hours at this concentration. As another example, ~~Botox~~BOTOX® at 1.0 ml 0.9% NaCl reconstitution has full activity, while reconstitution at 0.5 ml in 1% and 5% EtOH plus 0.9% NaCl produces solutions with full activity.

Please amend paragraph [0056] as follows:

[0056] Most preferably, the compositions are administered by or under the direction of a physician or other health care professional. They may be administered in a single treatment or in a series of periodic treatments over time. For transdermal delivery of botulinum toxin for the purposes mentioned above, a composition as described above is applied topically to the skin at a location or locations where the effect is desired. In embodiments were an aqueous botulinum toxin/carrier solution is applied directly to the skin, it is preferable to cover the treated area (e.g., with ~~Cetaphil~~CETAPHIL® moisturizer) or occlude the treated area with a barrier (e.g., Telfa), in order to prevent the solution from drying out, which would lead to a decrease in toxin activity. Because of its nature, most preferably the amount of botulinum toxin applied should be applied

with care, at an application rate and frequency of application that will produce the desired result without producing any adverse or undesired results. Accordingly, for instance, topical compositions of the invention should be applied at a rate of from about 1 U to about 20,000 U, preferably from about 1 U to about 10,000 U botulinum toxin per cm.sup.2 of skin surface. Higher dosages within these ranges could preferably be employed in conjunction with controlled release materials, for instance, or allowed a shorter dwell time on the skin prior to removal.

Please delete paragraph [0066] and replace it with the following paragraph:

[0066] The positively charged backbone was assembled by conjugating - Gly_3Arg_7 **(SEQ ID NO: 5)** to polylysine (MW 112,000) via the carboxyl of the terminal glycine to free amines of the lysine side chains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is conjugated to a - Gly_3Arg_7 **(SEQ ID NO: 5)**). The modified backbone was designated "KNR". The control polycation was unmodified polylysine (designated "K", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot.

Please amend paragraph [0067] as follows:

[0067] ~~Botox~~BOTOX® brand of botulinum toxin A (Allergan) was selected for this experiment. It has a molecular weight of approximately 150,000.

Please amend paragraph [0070] as follows:

[0070] Group labeled "JMW-7": 2.0 units of Btox-b per aliquot (i.e. 20 U total) and peptidyl carrier KNR at a calculated MW ratio of 4:1 were mixed to homogeneity and diluted to 200 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 1.8 ml of ~~Cetaphil~~CETAPHIL® lotion and aliquoted in 200 microliter portions.

Please amend paragraph [0071] as follows:

[0071] Group labeled "JMW-8": 2.0 units of Btox-b per aliquot (i.e. 20 U total) and K at a MW ratio of 4:1 were mixed to homogeneity and diluted to 200 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 1.8 ml of ~~Cetaphil~~CETAPHIL® and aliquoted in 200 microliter portions.

Please amend paragraph [0073] as follows:

[0073] Biotin visualization was conducted as follows. Briefly, each section was immersed for 1 hour in ~~NeutrAvidin~~ NEUTRAVIDIN® buffer solution. To visualize alkaline phosphatase activity, cross sections were washed in saline four times then immersed in NBT/BCIP (Pierce Scientific) for 1 hour. Sections were then rinsed in saline and photographed in entirety on a Nikon E600 microscope with plan-apochromat lenses.

Please delete paragraph [0077] and replace it with the following paragraph:

[0077] The positively charged backbone was again assembled by conjugating - Gly_3Arg_7 **(SEQ ID NO: 5)** to polylysine (MW 112,000) via the carboxyl of the terminal glycine to free amines of the lysine side chains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is conjugated to a - Gly_3Arg_7 **(SEQ ID NO: 5)**). The modified backbone was designated "KNR". Control polycation was unmodified polylysine (designated "K", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot. The same botulinum toxin therapeutic agent was used as in Example 1, and was prepared in the same manner. Samples were prepared as follows:

Please amend paragraphs [0078]-[0080] as follows

[0078] Group labeled "JMW-9": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and peptidyl carrier KNR at a calculated MW ratio of 4:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of ~~Cetaphil~~CETAPHIL®and aliquoted in 200 microliter portions.

[0079] Group labeled "JMW-10": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and K at a MW ratio of 4:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of ~~Cetaphil~~CETAPHIL® and aliquoted in 200 microliter portions.

[0080] Group labeled "JMW-11": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) without polycation was diluted to 600 microliters with phosphate buffered saline. The resulting

composition was mixed to homogeneity with 5.4 ml of ~~Cetaphil~~CETAPHIL® and aliquoted in 200 microliter portions.

Please amend paragraph [0083] as follows:

[0083] Mean digital abduction scores after single-time topical administration of botulinum toxin with KNR ("JMW-9"), K ("JMW-IO") or diluent without polycation ("JMW-H"), are presented in table 2 and illustrated in the representative photomicrograph of figure 2 below. The peptidyl carrier KNR afforded statistically significant functional delivery of the botulinum toxin across skin relative to both controls, which were comparable to one another. Additional independent repetitions (total of three independent experiments all with identical conclusions in statistically significant paralysis from topical botulinum toxin with KNR but not controls) of the present experiment confirmed the present findings and revealed no significant differences between topical botulinum toxin with or without K (i.e. both controls). Interestingly, the mice consistently ambulated toward a paralyzed limb (which occurred in 100% of treated animals and 0% of controls from either control group). As shown in Figure 2, a limb treated with botulinum toxin plus the control polycation polylysine or with botulinum toxin without polycation ("~~Botox~~ BOTOX® alone") can mobilize digits (as a defense mechanism when picked up), but the limbs treated with botulinum toxin plus the peptidyl carrier KNR ("Essentia Botox lotion") could not be moved.

Table 2 : Digital abduction scores 30 minutes after single-time topical application of botulinum toxin with the peptidyl carrier KNR ("JMW-9"), with a control polycation K ("JMW-IO"), or alone ("JMW-11").

Group	Mean	Std. Error
JMW-9	3.333	0.333
JMW-10	0.333	0.333
JMW-11	0.793	0.300

P=0.0351 (Significant at 95%)

Conclusions:

Please delete paragraph [0086] and replace it with the following paragraph:

[0086] The positively charged backbone was assembled by conjugating - Gly_3Arg_7 **(SEQ ID NO: 5)** to polyethyleneimine (PEI) MW 1,000,000 via the carboxyl of the terminal glycine to free amines of the PEI side chains at a degree of saturation of 30% (i.e., 30 out of each 100 lysine residues is conjugated to a - Gly_3Arg_7 **(SEQ ID NO: 5)**). The modified backbone was designated "PEIR" to denote the large nonpeptidyl carrier. Control polycation was unmodified PEI (designated "PEI", Sigma Chemical Co., St. Louis, Mo.) of the same size and from the same lot. The same botulinum toxin therapeutic agent was used as in example 1.

Please amend paragraph [0087] as follows:

[0087] Botulinum toxin was reconstituted from the ~~Botox~~BOTOX® product according to the manufacturer's instructions. In each case, an excess of polycation was employed to assemble a final complex that had an excess of positive charge as in delivery of highly negative large nucleotide complexes. A net neutral or positive charge prevents repulsion of the protein complex from highly negative cell surface proteoglycans and extracellular matrix. The botulinum toxin dose was standardized across all groups as was total volume and final pH of the composition to be applied topically. Samples were prepared as follows:

Please amend paragraphs [0088]-[0089] as follows:

[0088] Group labeled "AZ": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and the nonpeptidyl carrier PEIR in ultrapure form at a calculated MW ratio of 5:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of ~~Cetaphil~~CETAPHIL®and aliquoted in 200 microliter portions.

[0089] Group labeled "BA": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and PEI at a charge ratio of 5:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of ~~Cetaphil~~CETAPHIL®and aliquoted in 200 microliter portions.

Please amend paragraph [0092] as follows:

[0092] Mean digital abduction scores after single-time topical administration of botulinum toxin with ultrapure PEIR ("AZ"), or control polycation PEI ("BA"), are presented in table 3 and repetition presented as table 4 (single independent repetition for this experiment). The nonpeptidyl carrier PEIR afforded statistically significant functional delivery of botulinum toxin across skin relative to controls. As before, animals were observed to walk in circles toward the paralyzed limbs.

Table 3. Digital abduction scores 30 minutes after single-time topical administration of ~~Botox~~ BOTOX® with ultrapure PEIR ("AZ"), or control polycation PEI ("BA"). Mean and standard error are presented.

Group	Mean	Std. Error
BA	0.833	0.307
AZ	3.917	0.083

P=0.0002 (Significant at 99%)

Table 4. Digital abduction scores 30 minutes after single-time topical administration of Botox with ultrapure PEIR ("AZI"), or control polycation PEI ("BAI"). Mean and standard error are presented.

Group	Mean	Std. Error
BA1	0.333	0.211
AZ1	3.833	0.167

P=0.0001 (Significant at 99%)

Conclusions:

Please amend paragraph [0099] as follows:

[0099] The ~~Botox~~ BOTOX® reconstituting solution of sterile 0.9% sodium chloride (Abbott Laboratories, North Chicago, Ill.) plus 5% EtOH plus 5% short chained polyaspartate solution labeled A-3C (Donlar BioPolymer, Inc. Bedford Park, Ill.) was prepared (i.e., for every 1.0 milliliter solution, 900 microliters of sterile 0.9% sodium chloride plus 50 microliters of 100% EtOH plus 50 microliters of short chained polyaspartate solution). Kn21T was prepared at 1 milligram/milliliter concentration with 0.9% sodium chloride plus 5% EtOH (i.e., 500 microliters of Kn21T was aliquoted and 25 microliters of 100% EtOH was added). As used herein, Kn21T refers to a positively charged polylysine backbone having a molecular weight of 21,000 and TAT branching groups. 100 units of ~~Botox~~ BOTOX®(Allergan, Irvine, Calif.) was reconstituted with 1.0 milliliters of reconstituting solution using sterile 3 ml latex free syringe with $18_G1_{1/2}$ (Becton Dickinson & Co., Franklin Lakes, N.J.). The reconstituted ~~Botox~~ BOTOX®was carefully mixed by inversion 8 times. 200 units of ~~Botox~~ BOTOX®were used for each subject. The "Essentia Botox solution" was prepared with 200 units of Botox and Kn21T plus 5% EtOH (ie. 2.0 milliliters of ~~Botox~~ BOTOX® was added to 500 microliters of Kn21T plus 25 microliters of 100% EtOH) and sat at room temperature for 5 minutes for the complexes to form.

Please amend paragraph [0101] as follows:

[0101] The subject reclined on a table with protective covering around the eyes, face, and upper body. The treatment was applied evenly to the subject's forehead using a pipette and massaged into the skin in circular motion with fingers while wearing powder-free, nitrile gloves. The treatment area was covered with a thin layer of ~~Cetaphil~~CETAPHIL® moisturizing cream (Galderma, Fort Worth, Tex.) and incubated for 60 minutes. After 60 minute incubation, the treatment was removed with sterile gauze pads. The gauze pads and gloves were discarded in a biohazard bag.

Please amend paragraph [0111] as follows:

[0111] Kn21pr was prepared at 1 milligram/milliliter concentration with saline plus 5% EtOH (i.e., 500 microliters of Kn21pr was aliquoted and 25 microliters of 100% EtOH was added). As used herein, Kn21pr refers to a positively charged polylysine backbone with a

molecular weight of 21,000 and branching groups comprising protected oligoarginine. 100 units of ~~Botox~~ BOTOX® (Allergan, Irvine, Calif.) was reconstituted with 0.75 milliliters of 0.9% sodium chloride (Abbott Laboratories, North Chicago, Ill.) using sterile 3 ml latex free syringe with $18_G1_{1/2}$ (Becton Dickinson and Company, Franklin Lakes, N.J.). The reconstituted Botox.RTM. was carefully mixed by inversion 8 times. 200 units of ~~Botox~~ BOTOX® were used for each subject. The treatment solution was prepared with 200 units of ~~Botox~~ BOTOX® and Kn21pr plus 5% EtOH (i.e., 1.5 milliliters of ~~Botox~~ BOTOX® was added to 500 microliters of Kn21pr plus 25 microliters of 100% EtOH) and kept at room temperature for 5 minutes to allow the complexes to form. After a 5-minute incubation period, approximately 1.0 milliliters of 4% HPC (hydroxypropylcellulose) (with 1% EtOH) was added and mixed gently and thoroughly with a small metal spatula. The homogenous treatment solution was transferred into a 3 ml syringe and syringe tip cap. (Becton Dickinson and Company, Franklin Lakes, N.J.).

Please amend paragraph [00115] as follows:

[0115] Second comparison: Baseline vs. 4 weeks (See FIG. 8b). Sweat production (mg per 5 minutes) 4 weeks after axillary treatment (randomized by side) with kn21pr backbone alone (control) or kn21pr backbone plus 200 U ~~Botox~~ BOTOX® (ratio of treatment to control) presented in Table 6. Statistical analyses by Wilcoxon signed ranks using NPSS with P as noted and significance at $P<0.05$. (Pr T p=0.0217) [n=10].